To Sandwich Technetium: Highly Functionalized Bis-Arene Complexes [99mTc(6-arene)2]+ Directly from Water and [99mTcO4]−

Nadeem, Qaisar ; Meola, Giuseppe ; Braband, Henrik ; Bolliger, Robin ; Blacque, Olivier ; Hernández-Valdés, Daniel ; Alberto, Roger

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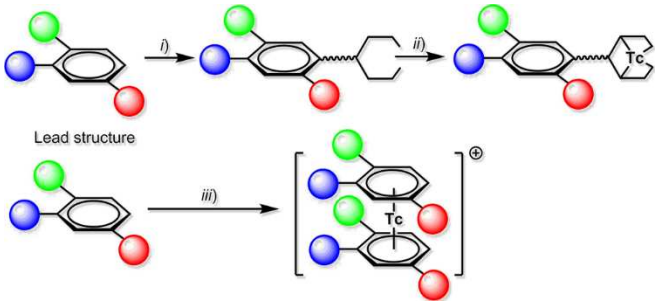
To sandwich technetium: highly functionalized bis-arene complexes $[^{99m}Tc(\eta^6\text{-arene})_2]^+$ directly from water and $[^{99m}TcO_4]^-$

Qaisar Nadeem, Giuseppe Meola, Henrik Braband, Robin Bolliger, Olivier Blacque, Daniel Hernández-Valdés and Roger Alberto*

Department of Chemistry, University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland
E-mail: ariel@chem.uzh.ch

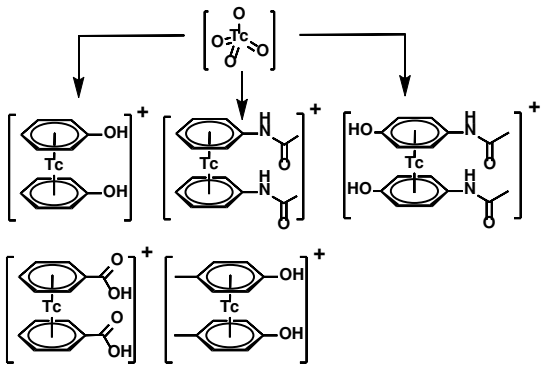
Abstract: The labeling of (bio)molecules with metallic radionuclides such as ^{99m}Tc demands conjugated, multidentate chelators. This is not always necessary since phenyl rings can directly serve as integrated, organometallic ligands. Bis-arene sandwich complexes are generally prepared by the Fischer-Hafner reaction. In extension, we show that $[^{99m}Tc(\eta^6\text{-}C_6R_6)_2]^+$ type complexes are directly accessible from water and $[^{99m}TcO_4]^-$, even with arenes incompatible with Fischer-Hafner conditions. To unambiguously confirm the nature of these unprecedented ^{99m}Tc complexes, their rhenium homologous have been prepared by substituting naphthalene ligands in $[Re(\eta^6\text{-}C_{10}H_8)_2]^+$ with the corresponding phenyls. The ease with which highly stable $[^{99m}Tc(\eta^6\text{-}C_6R_6)_2]^+$ are formed under standard labeling conditions enables a multitude of new potential imaging agents, based on commercial pharmaceuticals or lead structures.

In molecular imaging, targeting vectors or pharmaceutically active compounds are combined with radionuclides for the non-invasive detection of e.g. high proliferation rates, increased receptor densities and other abnormal physiological features.[1-2] The labeling of such vectors, often represented by comparably small molecules, relies typically on the direct formation of covalent bonds such as C-^{18}F or C-^{123}I.[3-4] Thereby, the basic lead structure of the molecule stays essentially intact. Many examples for such molecular imaging agents are in clinical application, [^{18}F]-FDG being probably the most prominent example. In contrast, the labeling with metallic radionuclides requests chelators for stabilizing the radionuclide against trans-metalation with ubiquitous and competing ligand sites in e.g. proteins (scheme 1, top).[5] These multidentate ligands are often bulky and their molecular weights may exceed the ones of the lead structures. DOTA for ^{68}Ga, DTPA or MAG3 for ^{99m}Tc or ^{111}In and other examples evidence this situation.[6-7] Since the labeled chelator bound to the vector substantially influences pharmacology, rational predictions for successful molecular imaging agents are often affected. Chelators are mandatory for main group metals but may be replaced by comparably small ligands for d-elements.
In this respect, cyclopentadiene (HCp) is small and the hypothesis that it can replace phenyls in pharmaceutically active lead structures has been shown in many examples, ferrocifen, an analogue of tamoxifen, being probably the most prominent one.[8-10] Our group corroborated the importance of cyclopentadiene conjugated to targeting molecules as pendent ligand or as integral part of lead structures in many examples.[11-15]
Cyclopentadiene or cyclopentadienyl is structurally only close to "natural" features in targeting molecules, in contrast to phenyls, which are ubiquitous in pharmaceuticals. Binding a metal center to such integrated or pendent phenyls would exclude conjugation of bulky chelators, if the η^6-bound metal were stable under biological conditions. The favorable biological behavior of *de novo* complexes with η^6-bound arenes is impressively delineated by the $[Ru^{II}(\eta^6\text{-cymene})]^{2+}$-based complexes, which pioneered this field.[16-20]



Scheme 1. Classic labelling procedure: i) derivatization of a lead structure with a chelator and ii) subsequent labelling with $[^{99m}TcO_4]$-. iii) direct labelling at a phenyl ring without bifunctional chelator with $[^{99m}TcO_4]$- in H_2O leads to "twinning" of the lead structures in sandwich complexes.

Arene complexes of rhenium and even more for technetium are scarcely investigated, despite their discovery some 60 years ago.[21-22] Being inspired by the opportunity of labeling molecules directly via phenyl groups and without chelators, we recently reported about the syntheses and properties of a variety of $[M(\eta^6\text{-arene})_2]^+$ (M=^{99}Tc, ^{99m}Tc, Re) complexes.[23-25] The syntheses followed classical Fischer-Hafner conditions, i.e. with $AlCl_3$ and Zn° or Al° as reductants. The extreme stabilities towards base, acid, air and water encouraged us to aim at a direct labeling of functionalized phenyls beyond the simple alkyl-bearing ones (scheme 1, bottom). Oxygen- or nitrogen functionalities are incompatible with Fischer-Hafner conditions. Furthermore, for binding e.g. pharmaceuticals to ^{99m}Tc, the reactions have to be performed in water, even more incompatible with Fischer-Hafner conditions.
We report here about a new method for preparing $[^{99m}Tc(\eta^6\text{-arene})_2]^+$ sandwiches with N- and O-functionalized arenes directly from water and $[^{99m}TcO_4]^-$ in moderate to very good yields and high radiochemical purities.



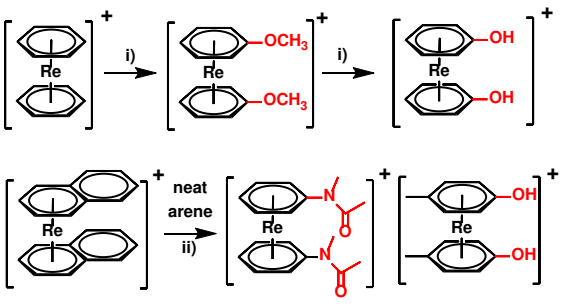
Scheme 2. Access to bis-arene $[^{99m}Tc(\eta^6\text{-}C_6R_6)_2]^+$ complexes in water from $[^{99m}TcO_4]^-$ with arenes incompatible with Fischer-Hafner conditions. Reducing agents depend on the arene but are typically Zn°/HCl, $H_3N{\cdot}BH_3$ or $Na[BH_4]$ and SDS for increasing the solubilities. More examples are given in the supplementary information.

The ease with which $[^{99m}Tc(\eta^6\text{-arene})_2]^+$ complexes are formed in water is surprising, given that arenes are not "typical" aqueous ligands. Reactions of $[^{99m}TcO_4]^-$ in saline, as eluted from the $^{99}Mo/^{99m}Tc$ generator, with different, functionalized arenes and Zn°, ammonia borane $H_3N{\cdot}BH_3$ or $Na[BH_4]$ as reductants, yield after 30-60min at 100° C directly and exclusively $[^{99m}Tc(\eta^6\text{-}C_6R_6)_2]^+$ as shown in scheme 2 and table S1 and figures S1-S19. Small amounts of dodecylsulfate (SDS) as surfactant were added to increase the solubilities of the arenes in water, which is a yield-limiting factor. Since all arenes are different, yields are variable but can be optimized individually. The radiochemical purity is always very high since the only products formed are $[^{99m}Tc(\eta^6\text{-}C_6R_6)_2]^+$ (see figure S22-S23). The once formed $[^{99m}Tc(\eta^6\text{-}C_6R_6)_2]^+$ complexes are very stable over a broad range of pH-values and up to temperatures of at least 160°C (μwave), thus, the reaction can be accelerated and yields optimized by increasing e.g. the temperature, if the functionalities on the arene rings resist such conditions . Due to the different physico-chemical properties of the functionalities on the phenyls, there is not one single reaction scheme for preparing the bis-arene complexes. The reductant and the pH-values are important but all the described arenes did label with either Zn°, $H_3N{\cdot}BH_3$ or $Na[BH_4]$ as reductants. Conditions can then be optimized within this scheme for a given arene.

Expectedly, reactions with all the arenes shown in scheme 2 and in the supplementary information with $[^{99}TcO_4]^-$ or $[ReO_4]^-$ did not yield any defined complexes under Fischer-Hafner conditions (except, 4-phenylmorpholine, in 2-4 % yield of only Re-complex, scheme S2 in SI). Oxygen - or nitrogen containing functionalities reacted rapidly with $AlCl_3$, leading to decomposition, side reactions and deactivation of $AlCl_3$ and other Lewis acids. Since only comparison of the HPLC retention times of fully characterized ^{99}Tc or Re complexes with the products of the 99mTc reactions allows for assessing their authenticity, another approach for rhenium or ^{99}Tc had to be found (vide infra).

Two options for the preparation of model rhenium complexes are principally feasible; i) post-modification of e.g. $[Re(\eta^6\text{-}C_6H_6)_2]^+$ or ii) substitution of labile ligands in a ReI precursor (scheme 3).

Since i) becomes easily multi-step with more complex side chains, we proceeded with ii). Examples for this procedure exist for group 8 M^{II} precursors in particular, typically starting from $[M(\eta^6\text{-naphthalene})_2]^{2+}$ or $[M(\eta^5\text{-Cp})(sol)_3]^+$ (M=Fe, Ru, sol = $NCCH_3$) or similar compounds.[26-29]



Scheme 3. Path i) post functionalization of $[Re(\eta^6\text{-}C_6H_6)_2]^+$ to yield the bis-phenol complex $[Re(\eta^6\text{-}C_6H_5\text{-}OH)_2]^+$, and path ii) substitution of naphthalene with a functionalized arene.

The reaction of $[Re(\eta^6\text{-naphthalene})_2]^+$ with the neat arenes, e.g. paracetamol or *p*-cresol, at high temperatures lead to the desired products $[Re(\eta^6\text{-}C_6R_6)_2]^+$, albeit in variable yields. Separation by preparative HPLC and subsequent crystallization gave the pure products, which could now be compared by analytical HPLC with the compounds formed with ^{99m}Tc. Figure 1 shows the X-ray structures of $[Re(\eta^6\text{-}C_6H_5\text{-}OH)_2]^+$ and $[Re(\eta^6\text{-paracetamol})_2]^+$ and figure 2 the HPLC comparison of the respective rhenium (UV/vis detector) and the ^{99m}Tc complex with paracetamol (γ-detector). The almost identical retention times assess the authenticity of the ^{99m}Tc complex, which is identical to the fully characterized rhenium homologue. Further examples are given in the supplementary material.

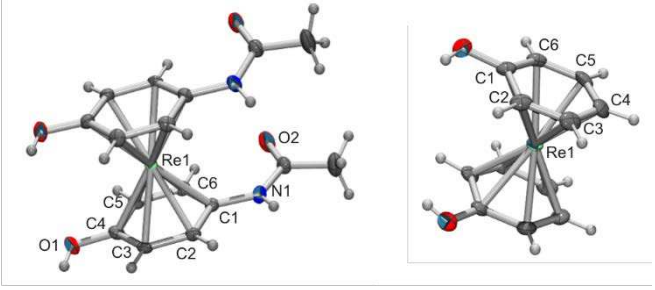


Figure 1. ORTEP representations of $[Re(\eta^6\text{-paracetamol})_2]^+$ (left) and $[Re(\eta^6\text{-}C_6H_5\text{-}OH)_2]^+$ (right). Ellipsoid are drawn on the 50% level.

Arenes are thus directly labeled, even if they are functionalized with potentially coordinating groups such as amino- or hydroxyl functions, this is a core message from this report. This mode of reactivity is unprecedented and has not been considered at all in labeling procedures so far. The formation of [M(η6-arene)2]+ complexes may well account for unidentified side products formed during standard labeling reactions of molecules comprising phenyl groups.

The reaction mechanisms consist of an in total 6e$^-$ reduction from Tc^{VII} to Tc^{I}, in the transfer of eight protons for the formation of four H_2O molecules together with the coordination of the two arenes. Many competing ligands such as halides, water and electrolyte anions are present in solution. Still, the arene as an atypical ligand in water coordinates to the once reduced ^{99m}Tc center.

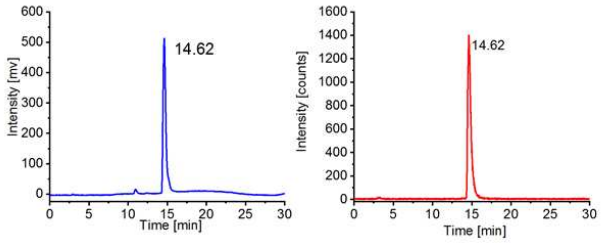
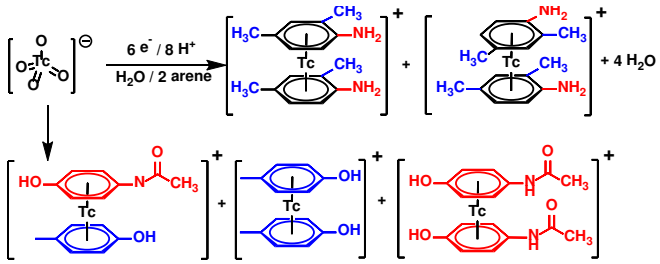


Figure 2. HPLC trace (UV/vis detection, left) of [Re(η^6-paracetamol)$_2$]$^+$ and [^{99m}Tc(η^6-paracetamol)$_2$]$^+$ (γ-detection, right) confirm the structure of the ^{99m}Tc complex.

Multiple elementary steps must be involved in this highly complex reaction scheme. We can only speculate about the mechanism but three stereochemical features should still be noted; bis- or multi-functionalized phenyls with ortho-, meta- or multi-substituents will yield two stereoisomers as observed with 2,4-dimethylaniline (scheme 4, upper line for ^{99m}Tc complex, Scheme S7 for Re-complex). The HPLC γ-trace of the ^{99m}Tc complex and the UV/vis trace of the Re homologue show one peak but the NMR spectrum clearly evidenced two compounds (Figures S14 and S33). In case of chiral side chains, three stereoisomers are expected due to the prochirality of the ^{99m}Tc center. A reaction with two different arenes of about the same reactivities towards bis-arene complex formation and solubilities, *p*-cresol and paracetamol, gave the three complexes [^{99m}Tc(η^6-*p*-cresol)$_2$]$^+$, [^{99m}Tc(η^6-paracetamol)$_2$]$^+$ and the mixed arene complex [^{99m}Tc(η^6-*p*-cresol)(η^6-paracetamol)]$^+$ (scheme 4 lower line and figures S2-S6). As shown in figure 3, these complexes are clearly discernible on the HPLC analysis. We note that the traces remain unchanged when the solution was kept in air over night, corroborating the stabilities of bis-arene complexes of ^{99m}Tc. Whereas these expected and actually found stereochemical or stochastic features serve as indirect proofs for the authenticities of [^{99m}Tc(η^6-arene)$_2$]$^+$ complexes, only a comparison with a fully characterized ^{99}Tc or Re complex will confirm unambiguously their identities .



Scheme 4. Stereochemical and stochastic features for multi-functionalized arenes and unsymmetrical products when labeling two different arenes the same time with ^{99m}Tc.

In conclusion, sandwich complexes of the bis-arene-type can be prepared with ^{99m}Tc directly from [^{99m}TcO$_4$]$^-$ and in water in a convenient procedure. Potentially, technetium can be sandwiched with many organic molecules, comprising phenyls without the need for conjugating additional chelators. Exact conditions need to be optimized from phenyl to phenyl but the given reaction schemes can be applied to a wide variety of phenyls. These general routes allow access to a multitude of new molecular imaging agents and are applicable to the labeling of biomolecules. We emphasize that unidentified side products in other standard labeling procedures may well account for sandwich-type ^{99m}Tc complexes.

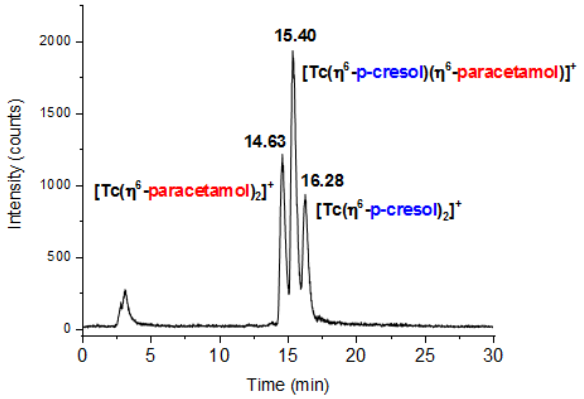


Figure 3. The reaction of [^{99m}TcO$_4$]$^-$ with two different arenes (p-cresol and paracetamol) leads to the three clearly discernible sandwich complexes [^{99m}Tc(η^6-p-cresol)$_2$]$^+$, [^{99m}Tc(η^6-paracetamol)$_2$]$^+$ and the mixed arene complex [^{99m}Tc(η^6-p-cresol)(η^6-paracetamol)]$^+$

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Keywords: Sandwich complexes • Technetium • Rhenium • Molecular imaging • Screening

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